REMARKS

Claims 1-9 and 12-58 are all the claims pending in the application. Claims 19-58 are withdrawn from consideration. Claims 1-9 and 12-18 are rejected.

I. Claims 1-9 and 12-18 Are Definite Under 35 U.S.C. § 112, Second Paragraph

A. At page 3 of the Office Action dated December 8, 2006, the Office rejects claims 1-9 and 12-18 under 35 U.S.C. § 112, second paragraph as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as their invention. At page 3 of the Office Action dated December 8, 2006, the Office asserts that one of reasonable skill in the art is unaware of the means by which to measure the dissociation constant of claim 1 because Applicants have allegedly failed to "provide a definite standard."

Applicants respectfully disagree and traverse the rejection. Applicants herein incorporate Applicants' prior arguments during an Interview dated September 1, 2005, in which counsel directed the Office to teaching in the specification. The Office construed the teachings as being "experimental conditions for meas.[uring] a binding constant." Interview Summary, September 1, 2005, page 1. Applicants also incorporate the dissociation constant standard teachings provided in the Reponses filed on October 31, 2005, December 21, 2005, and September 22, 2006, which are fully incorporated herein. Further, one of ordinary skill in the art would know how to measure a dissociation constant. Assuming *arguendo* that one of ordinary skill in the art did not understand how to measure a dissociation constant, which he would, Applicants direct the Office to the teachings in Applicants' U.S. patent application Publication Nos. 2003/0153026, 2003/0134346 and 2003/0130167. These applications have been incorporated by reference into the present application and provide standards for measuring dissociation constants. Therefore, based on Applicant's teachings and the knowledge of one of ordinary skill in the art, standards for measuring dissociation constants are routine and apparent.

Applicants kindly request reconsideration and withdrawal of the indefiniteness rejection.

B. At page 3 of the Office Action dated December 8, 2006, the Office asserts that step (a), which recites "said fusion protein has a dissociation constant of at least 1 mM toward said

analyte" does not match in scope with step (c) which recites, "said analyte is bound to said functional mutant periplamsic glucose-galactose binding protein."

Without agreeing with the Office, and to better capture the envisioned commercial embodiments, Applicants amend claim 1 step (c) to recite, "measuring the luminescence value of said fusion protein at said first emission wavelength, when said analyte is bound to said functional mutant periplasmic glucose-galactose binding protein of said fusion protein, wherein said fusion protein has a dissociation constant of at least 1mM towards said analyte."

Applicants assert that the amendment renders moot the rejection. Accordingly, Applicants kindly request reconsideration and withdrawal of the indefiniteness rejection.

C. At page 3 of the Office Action dated December 8, 2006, the Office asserts that recitation of "the" in step (d) of claim 1 is grammatically awkward.

Without agreeing with the Office and solely to advance prosecution, Applicants amend the claim and overcome the rejection.

Applicants kindly request reconsideration and withdrawal of the indefiniteness rejection.

D. At page 3 of the Office Action dated December 8, 2006, the Office alleges that the term "alexa" as recited in claim 18 is indefinite, since the identity of alexa is not clear.

Applicants disagree. One of ordinary skill in the art would recognize the meaning and identity of alexa. For example, in the Response dated September 2, 2005, Applicants provided Nanchuk-Voloshinal *et al.* Applicants note that alexa is not considered a trademark anywhere in the provided reference. Since it is possible, under U.S. trademark law, that indicia qualify as trademarks once registrable, alexa may be a trademark by actual use, but not *per se*.

Without agreeing with the Office, and solely to advance prosecution, Applicants amend claim 18 to identify alexa as a trademark by actual use. Applicants assert, however, that Alexa refers to a product, not a source. Applicants further assert that the amendment in no way impacts the material features of the claims.

Applicants kindly request reconsideration and withdrawal of the indefiniteness rejection.

II. The Claims are Patentable Under 35 U.S.C. § 103(a)

A. At page 3 of the Office Action dated December 8, 2006, the Office alleges that claims 1-9, 12-13 and 17-18 are obvious under 35 U.S.C. § 103(a) over Hellinga & Looger, in view of Romoser *et al.*

The Office alleges that Hellinga & Looger disclose a method for quantifying an analyte in a sample. The Office admits that Hellinga & Looger do not describe a detection scheme based on resonance energy transfer incorporating a labeling moiety and fluorescence protein. The Office alleges that Romoser *et al.* describe a detection scheme based on resonance energy transfer incorporating a labeling moiety and fluorescence protein.

Applicants disagree. To establish a *prima facie* case of obviousness, the Office must prove that each and every element of the invention is contained within the cited references. Hellinga & Looger do not disclose Applicants' fusion proteins. In fact, the proteins of Hellinga & Looger appear to be limited to periplasmic proteins and metal-binding proteins or redox-active proteins. For example, at paragraph 29 of Hellinga & Looger, the reference states, "the reporter group can be present as a fusion between the protein and a metal binding domain (for instance, a small redox-active protein such as a cytochrome." In addition, it would be apparent to one of ordinary skill in the art that the fusion proteins of Hellinga & Looger are useful for constructing an amperometric or potentiometric sensor, not the Applicants' invention.

Futhermore, even assuming *arguendo* that the combination of Hellinga & Looger and Romoser *et al.* teaches each and every element of the claimed invention, which it does not, the Office does not provide a reason as to why one of ordinary skill in the art to would combine the elements in the references in the manner claimed. Applicants herein incorporate arguments previously submitted in the Response filed on September 22, 2006, page 11, which clearly establish at least that the analyte-binding protein used in the Romoser *et al.* system is so different from Applicants' periplasmic binding proteins that the differences would render Romoser *et al.* useless for a PBP-based detection scheme. Hellinga & Looger teach away from the Applicants' invention, because their fusion proteins are useful for constructing an amperometric or potentiometric sensor, not a PBP-based detection scheme. Hellinga & Looger, paragraph 29.

Romoser *et al.* states that the fluorescence at wavelength 510 nm is actually reduced when FRET is used, stating "fluorescent filters…were required [in the FRET system] to obtain adequate fluorescent signal."

In addition, there is no reasonable expectation of success that a skilled artisan could prepare a periplasmic binding protein fusion protein that utilizes FRET as a detection scheme by simply modifying Hellinga & Looger and Romoser *et al.*, because the references and the invention are incompatible. Applicants herein incorporate arguments previously submitted in the Response filed on September 22, 2006, page 11, which clearly establish that Romoser *et al.* discloses that two fluorescent proteins combine to provide *less* fluorescence during FRET with calmodulin. It is therefore impossible that a skilled artisan would reasonably expect that one could arrive at Applicants' periplasmic binding protein fusion protein that utilizes FRET as a detection scheme, based on the combination of Romoser *et al.* with Hellinga & Looger.

The Office has not established a *prima facie* case of obviousness because the cited references fail to teach each and every element of the claims. But even if the references teach all the limitations of the claimed invention, Applicants' invention is not rendered obvious by the cited references, because the Office does not provide a reason as to why one of ordinary skill in the art would combine the elements in the references in the manner claimed. Furthermore, the Office has not pointed to any reasonable expectation of success that a skilled artisan could prepare a periplasmic binding protein fusion protein that utilizes FRET as a detection scheme by simply modifying Hellinga & Looger and Romoser *et al.*

Applicants kindly request reconsideration and withdrawal of the obviousness rejection.

B. At page 5 of the Office Action dated December 8, 2006, the Office rejects claims 14-16 under 35 U.S.C. 103(a), alleging that they are unpatenable over Hellinga & Looger, and Romoser *et al.*, in light of Tsien & Campbell.

The Office alleges that Hellinga & Looger disclose a method for quantifying an analyte. The Office further alleges that Tsien & Campbell disclose use of DsRed2, including C119, and DsRed (C117E) as a member of a donor acceptor pair for fluorescence resonance energy transfer. The Office alleges it would have been obvious for one of ordinary skill in the art to

modify the method of Hellinga & Looger and Romoser *et al.* by using DsRed2 as disclosed in Tsien & Campbell.

Applicants herein incorporate prior arguments made in response to this rejection. Specifically, the arguments set forth in the Response filed May 9, 2005, September 2, 2005; October 31, 2005; December 21, 2005; and September 22, 2006 are incorporated herein in their entirety.

Moreover, the Office fails to establish a *prima facie* case of obviousness, since Tsien & Campbell does not disclose mutation of C119 of dsRed2, nor do they characterize such a mutation as beneficial for emission. It would therefore not have been obvious to one of ordinary skill in the art that particular cysteine mutations would be needed in the process of labeling a GFP or dsRed fluorescent fusion protein to obtain useful sensor output.

As shown in the attached 37 C.F.R. § 132 Declaration of Dr. Pitner, mutation of a reactive cysteine, namely C119, within the fluorescent protein domain of the fusion protein to achieve selective labeling of only the PBP cysteine with the desired labeling moiety is useful for obtaining meaningful changes in FRET of the acrylodan-labeled fusion protein. Applicants alone discovered that the presence of C119 in the wild-type dsRed2 - GGBP fusion protein leads to overlabeling with the acrylodan dye, thus resulting in useless sensor values. Applicants, however, find that introduction of the C119A mutation into the dsRed2 fluorescent protein sequence results in measurable changes in FRET of the acrylodan-labeled fusion protein corresponding to addition of analyte glucose. One of skill in the art would not have envisioned that mutation of C119, or any other cysteine, would lead to predictable changes in FRET of the acrylodan-labeled fusion protein. More specifically, nothing in the interrelated teachings of the references of record would lead one or ordinary skill in the art to mutate C119 of dsRed2. Tsien & Campbell do not disclose mutation of C119 of dsRed2, nor do they characterize such a mutation as beneficial for emission or for FRET measurement. See MPEP §716.02(e).

Since the cited references fail to teach each and every limitation of the claims, and because the Office has not identified a reason that would have prompted a person of ordinary

skill in the relevant field to combine the cited references, the combination of cited references cannot render obvious Applicants' invention.

Applicants kindly request reconsideration and withdrawal of the obviousness rejection.

Should the Examiner believe that further discussion of any remaining issues would advance the prosecution, he or she is invited to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,

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